

CLAIMS

1. A gene encoding two antibody variable domains, wherein the two antibody variable domains are connected by a linker comprising a restriction enzyme site.
5
2. The gene of claim 1, wherein the linker comprises two or more restriction enzyme sites.
3. The gene of claim 1 or 2, wherein one of the two antibody variable domains is a heavy chain variable domain and the other is a light chain variable domain.
10
4. The gene of any one of claims 1 to 3, wherein the two antibody variable domains are connected by a long linker.
5. A gene encoding two antibody variable domains, where both ends comprise a restriction enzyme site.
- 15 6. The gene of claim 5, wherein one of the two antibody variable domains is a heavy chain variable domain and the other is a light chain variable domain.
7. The gene of claim 5 or 6, wherein the two nucleotides encoding the two antibody variable domains are connected with a long linker.
- 20 8. A gene encoding four antibody variable domains, wherein the gene comprises a restriction enzyme site between the first and second antibody variable domains, and between the third and fourth antibody variable domains.
9. The gene of claim 8, wherein the first and second antibody variable domains are connected with a short linker, the third and fourth domains are connected with a short linker, and the second and third antibody variable domains are connected with a long linker.
25
10. The gene of claim 8 or 9, wherein the four antibody variable domains are a heavy chain variable domain and a light chain variable domain directed against a first antigen, and a heavy chain variable domain and a light chain variable domain directed against a second antigen.
30
11. The gene of claim 10, wherein the four antibody variable domains are comprised in the order: a light chain variable domain against the first antigen, a heavy chain variable domain directed against the second antigen, a light chain variable domain against the second
35

antigen, and a heavy chain variable domain against the first antigen.

12. A method for constructing a gene encoding a bispecific single chain diabody, wherein the method comprises:

(a) treating the gene of any one of claims 1 to 4 with a restriction enzyme;

(b) treating the gene of any one of claims 5 to 7 with a restriction enzyme; and

(c) inserting the gene constructed in step (b) into the gene constructed in step (a).

13. A peptide encoded by a gene of any of claims 1 to 11.

14. An antibody library comprising a gene of any of claims 1 to 11.

15. A method for constructing an antibody library or expression vector, wherein the method comprises:

(a) constructing an antibody phage library in which a light chain variable domain and a heavy chain variable domain, both directed against a first antigen, are connected with a long linker comprising a restriction enzyme site;

(b) constructing an antibody phage library in which a light chain variable region and a heavy chain variable domain, both directed against a second antigen, are connected with a long linker at one end, where the other ends comprise a restriction enzyme site;

(c) treating the phage libraries constructed in steps (a) and (b), or genes comprising the variable domains prepared from these phage libraries, with a restriction enzyme; and

(d) performing ligation of the fragments obtained from the above treatment to construct a fragment in which the heavy and light chain variable domains against the second antigen are inserted between the light and heavy chain variable domains against the first antigen.

16. A method for constructing an antibody library or expression vector, wherein the method comprises:

(a) treating the gene of any one of claims 1 to 4 with a restriction enzyme;

(b) treating the gene of any one of claims 5 to 7 with a restriction enzyme; and

(c) inserting the gene constructed in step (b) into the gene

constructed in step (a).

17. A method for constructing an antibody library or expression vector, wherein the method comprises:

5 (a) constructing an antibody phage library in which a light chain variable domain and a heavy chain variable domain, both against an antigen, are connected with a long linker comprising two or more restriction enzyme sites;

(b) treating the above phage library, or genes comprising variable domains prepared from the phage library, with a
10 restriction enzyme; and

(c) performing self-ligation of the fragments obtained above to shorten the linker between the variable domains.

18. An expression vector comprising a gene of any one of claims 1 to 11.

15